Reference: *Biol. Bull.* **171**: 208–216. (August, 1986)

EFFECTS OF OPIOIDS AND ANTAGONISTS ON THE RATE OF SEA URCHIN SPERM PROGRESSIVE MOTILITY

L. CARIELLO[1], L. ZANETTI[1], A. SPAGNUOLO[1], AND L. NELSON[2]*

[1]*Stazione Zoologica, Naples, Italy;* [2]*Department of Physiology, Medical College of Ohio, Toledo, Ohio 43699; and Marine Biological Laboratory, Woods Hole, Massachusetts*

Abstract

Opioids exert a profound effect on the motility of sea urchin sperm cells suspended in artificial seawater (ASW). With prolonged exposure to narcotic agents, the rate of progression increased in a time- and dose-dependent manner up to an optimum concentration. Methionine enkephalin acetate (metenkephalin) caused a doubling in the rate of progression while the opiate antagonist, (−)-naloxone caused the sperm cells to increase their rate of forward motion by up to 75% above the control rate. The metenkephalin increase occurred at lower concentrations than the naloxone optimum. In combination, a subthreshold dose of (−)-naloxone, completely abolished the stimulatory effects of metenkephalin. Plus-naloxone, the inactive isomer of (−)-naloxone did not have any effect by itself on sea urchin sperm motility nor did it alter the metenkephalin-induced effect. Even though the magnitude and the nature of the sperm cells' responses to the opioids and antagonists appear somewhat atypical compared to the responses of neurobiological systems, the lack of effect of (+)-naloxone confirms the specificity of the response and is consistent with the presence of opioid receptors in the sperm cell.

Introduction

Neuroactive agents cause sperm cells to alter their swimming behavior and, in some cases may affect their fertilizing capacity (Atherton *et al.,* 1978; Bavister *et al.,* 1979; Cornett and Meizel, 1978; Nelson, 1978; Sastry *et al.,* 1981).

The sperm cells of mussels, sea urchins, and starfish modulate their behavior when exposed to cholinergic agents (Nelson, 1978). Spermatozoa of vertebrate species from rams (Stewart and Forrester, 1978), bulls (Egbunike, 1982), boars (Sekine, 1951), and rabbits (Bishop *et al.,* 1976) to humans (Zeller and Joel, 1941; Sastry *et al.,* 1981) also tested positively for the presence of acetylcholine, its receptor, acetylcholinesterase, and choline acetyltransferase.

The rate of propulsion of sea urchin spermatozoa varies biphasically both when exposed to acetylcholine and to nicotine (Nelson, 1972). The presence of a nicotinic receptor in the sea urchin sperm was confirmed when it was shown that both d-tubocurarine (Nelson, 1973) and α-bungarotoxin (Nelson, 1976) inhibited their motility. Low concentrations of decamethonium, which interacts with the cholinergic receptor at neural synapses and neuromuscular junctions, caused a slight transitory increase in the rate of motile progression but depressed the motility at slightly higher concentrations (Nelson, 1973).

Cholinergic agents have been postulated to affect the transmembrane and intracellular transport of calcium into and within the sperm cell (Nelson, 1978). This

Received 10 March 1986; accepted 6 May 1986.

* Address for correspondence and reprint requests.

interdependence resembles the acetylcholine-calcium relationship in brain cell synaptosomes demonstrated by the inhibition of the release of acetylcholine due to the blocking of calcium uptake when the synaptosomes are incubated in the presence of morphine (Sanfacon *et al.*, 1977). The action of β-endorphin on rat brain synaptosomes was even more potent (Guerrro-Munoz *et al.*, 1979).

It was found that after a delay period, morphine-treated spermatozoa of the Mediterranean sea urchins, *Arbacia lixula, Paracentrotus lividus,* and *Sphaerechinus granularis,* appeared to swim more rapidly and for longer periods than untreated control cells under microscopic examination (Cariello and Nelson, 1984). The present experiment was designed to test whether the opioid effect on sperm cell behavior could be interpreted in terms of the effects of the narcotics on the brain synaptosome model.

Materials and Methods

To quantify the effects of the treatment, spermatozoa of the purple sea urchin, *Arbacia punctulata,* (the sea urchins, kept in running seawater aquaria were replenished several times weekly as needed during June, July and August) were aligned and oriented under gentle centrifugation in the horizontal rotor of an IEC Model CL centrifuge. Male sea urchins were induced to spawn by intracoelomic injection of 0.5 *M* KCl. One-tenth ml of the concentrated sperm cells was diluted in 20 ml of artificial seawater (ASW) (The turbidity of the suspension was adjusted to an optical density of 0.5–0.7, equivalent to 7–9 $\times$ 10^6 cells/ml.) One liter of ASW contains: NaCl, 24.72 g; KCl, 0.67 g; $CaCl_2 \cdot 2H_2O$, 1.36 g; $MgCl_2 \cdot 6H_2O$, 4.66 g; $MgSO_4 \cdot 7H_2O$, 6.29 g; and $NaHCO_3$, 0.18 g. Two ml of the sperm cell suspension were pipetted into each of 6 round, 1-cm diameter cuvettes. The control cuvette contained 0.2 ml of ASW; the second cuvette contained 0.2 ml of 10% formaldehyde; the remaining 4 cuvettes contained 0.2 ml of a given concentration of the test reagent. Each cuvette was inverted 2$\times$ to disperse the contents. The suspensions were preincubated for 0, 5, 10, and 15 minute periods at room temperature (22–25°C) and the sperm cells were aligned at 120 $\times$ *g* for 4 min. This centrifugal force orients the cells while displacing the formaldehyde-killed and non-motile cells only minimally or not at all (Nelson, 1972). Treated cells then swim more rapidly or more slowly than the control cells toward the bottom of the cuvettes and the optical densities (O.D.) at 540 nm are measured in a Bausch and Lomb model 340 spectrophotometer, before and after orientation. Readings were corrected for any displacement of the killed cells and forward motility was expressed as percent of the change in O.D. of the control suspension normalized to 100 (Nelson, 1972). The effect of each concentration of the opioid or antagonist was determined on at least six different samples for each incubation period. The sperm cells were exposed to methionine enkephalin acetate (Sigma) over a concentration range of 0.0016 to 1.23 m*M* and to (−)-naloxone-HCl (Endo) over a range of 0.0016 to 4.87 m*M* (Figs. 1, 2). The effect of competition between the opioid and the antagonist was tested at a fixed concentration of 0.005 m*M* naloxone over the entire concentration series of metenkephalin (Fig. 3). Plus-naloxone, (kindly supplied by NIDA), the enantiomer of (−)-naloxone, which presumably has much less ability to interact with the opiate receptor (Iijima *et al.*, 1978), was diluted to concentrations of 0.0016 m*M* to 1.09 m*M* in ASW. Plus-naloxone was tested both alone and in combination with the metenkephalin for the sea urchin sperm motility responses (Fig. 4).

Results

The *Arbacia* sperm responded maximally to metenkephalin at a concentration between 0.1 and 0.2 millimolar whether or not the cells had been preincubated in the

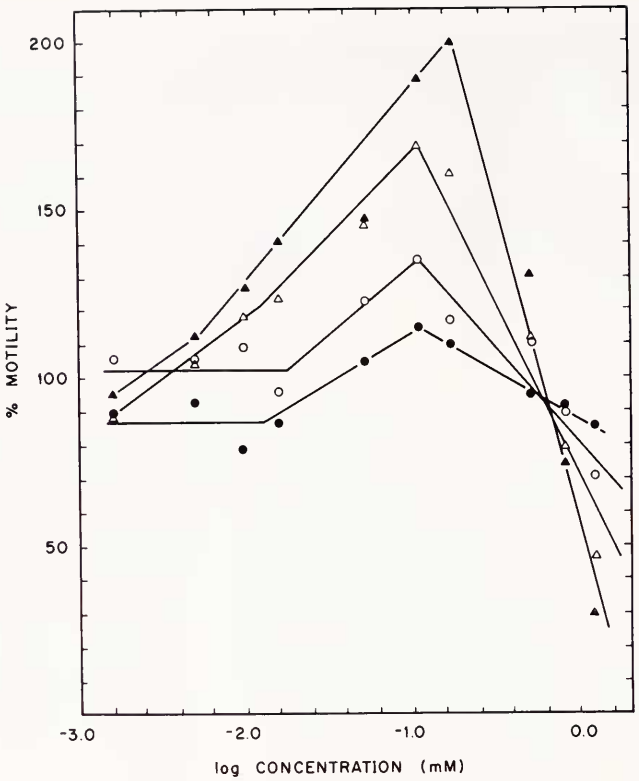

FIGURE 1. Motility of *Arbacia* sperm suspended in ASW containing 0.0016 to 1.23 m*M* metenkephalin. Abscissa: log conc. in m*M*. Ordinate: percent change in motility (control taken as 100%) after orientation at 120 × *g*. Symbols represent periods of preincubation before orientation. Solid circles, 0 min; open circles, 5 min; open triangles, 10 min; solid triangles, 15 min.

opioid before centrifugal orientation. While the brief exposure of the "zero-time" cells during centrifugation elicited only about a 15% increase in progressive rate at that concentration, less than 5 micromoles per liter appeared to suffice as a threshold concentration for the metenkephalin preincubated for 15 minutes before alignment. Concentrations of the opioids greater than 1 mM/l ASW were inhibitory (Fig. 1).

The response to the opiate antagonist, (−)-naloxone, began to develop at about 25 micromolar while maximal stimulation occurred at 0.5 millimolar and lesser increases in forward motility appeared at slightly higher concentrations, inhibition occurring at 2 millimolar and above (Fig. 2). Metenkephalin caused a doubling at its optimum while naloxone stimulated the sperm to a 75% increase over the control rate at its optimum when the sperm cells were preincubated for 15 minutes. Nearly complete inhibition with 15 minutes exposure occurred at about 1 millimolar metenkephalin but it required about 5 times that amount of (−)-naloxone to cause nearly complete cessation of movement.

Since both metenkephalin and (anomalously), (−)-naloxone appeared to exert delayed stimulatory effects on the spermatozoa to somewhat different though overlapping concentrations, it was important to test for interactive effects of the opioid and the so-called antagonist. This is illustrated in Figure 3, a composite of the graphs

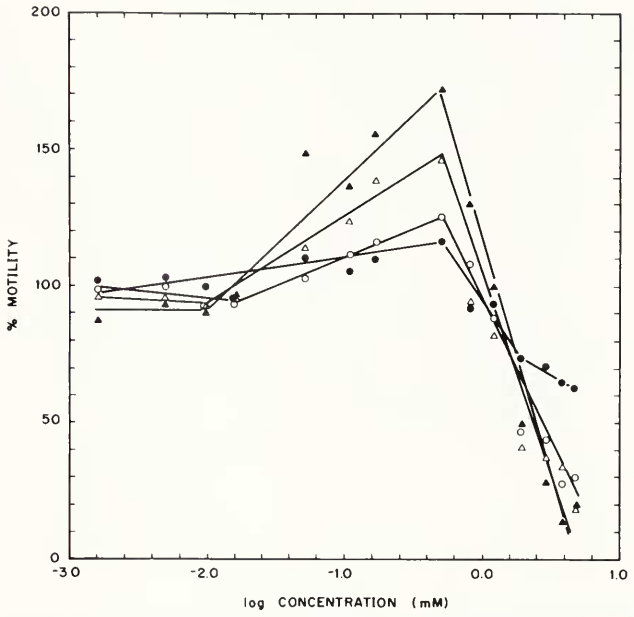

FIGURE 2. Motility of *Arbacia* sperm suspended in ASW containing 0.0016 to 4.87 mM (−)-naloxone-HCl. Abscissa: log conc. in m*M*. Ordinate: percent change in motility after 4 min orientation at 120 × *g*. Symbols, as in Figure 1.

of the results of the 15-minute preincubation periods for metenkephalin, open circles; (−)-naloxone, solid circles; and the mixture of the two compounds, open squares. In the combined drug study, the sperm cells were incubated at room temperature in suspensions containing about 1/5 the minimally effective concentration of (−)-naloxone, 5 μmole/l, over the whole concentration range of metenkephalin assayed (from 0.0016 to 1.23 millimolar). Alone, metenkephalin depressed motility below the control rate at about 0.8 m*M* and increased the motile rate by about 15% at 7 μ*M*. The subliminal concentration, 5 μ*M*, of (−)-naloxone completely blocked all stimulatory responses to the metenkephelin, although the rate of decline in motility induced by supraoptimal concentrations of either the opioid or antagonist alone was less pronounced in the combined drug medium (Fig. 3).

Since the (−)-naloxone exhibited atypical pharmacological action in the sea urchin sperm system, the inactive enantiomer, (+)-naloxone, was tested for its effects in the motility assay. Figure 4 shows the sperm cell responses to this drug, both alone and in combination with metenkephalin. By itself, (+)-naloxone (triangles) had no effect on motility at concentrations between micromolar and millimolar. Similarly, the increased motility due to metenkephalin (circles) was unaffected when the incubation medium contained 5 micromolar (+)-naloxone along with the metenkephalin (x's), in contrast to the reversing effect of 5 micromolar (−)-naloxone on metenkephalin shown in Figure 3. The data in Figure 4 depict the effects after a 15 min incubation. Similarly, the curve for the (−)-naloxone alone (squares) measured concurrently shows comparable characteristics with the previous experiment in Figure 3 (The data for Figs. 1, 2, and 3 were collected in July, 1984, those for Fig. 4 in July, 1985.)

In preliminary experiments morphine sulfate exerted a depressant effect on *Sphaerechinus* sperm during brief exposure, but following prolonged incubation, up to 45

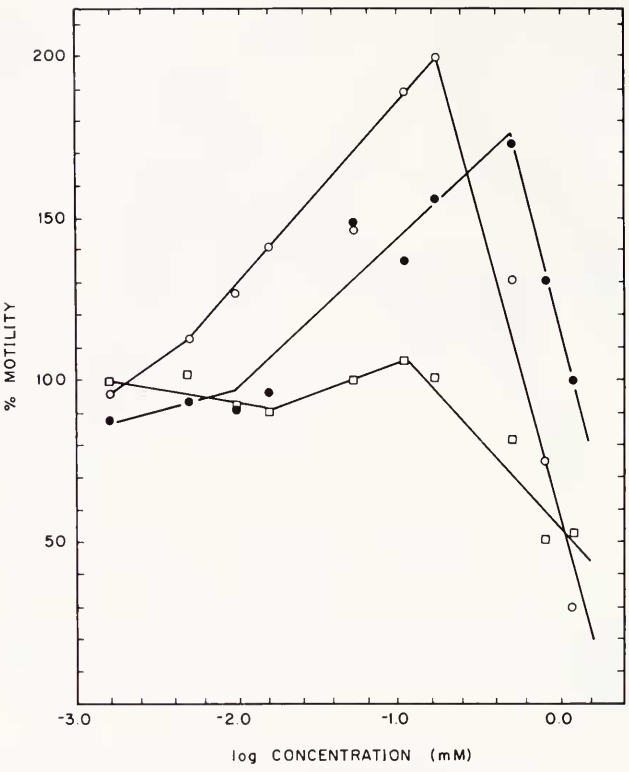

FIGURE 3. Drug interaction effect on *Arbacia* sperm. Incubation mixture contains (−)-naloxone at a fixed concentration of 0.005 m*M* and varying amount of metenkephalin. Ordinate: percent change in motility after 15 min incubation. Abscissa: log conc. in m*M*. Open squares 0.005 m*M* (−)-naloxone and 0.0016 to 4.87 m*M* metenkephalin; open circles, metenkephalin alone, 0.0016 m*M* to 1.23 m*M;* solid circles, (−)-naloxone alone 0.0016 to 4.87 m*M*. Note that 0.005 m*M* (−)-naloxone completely blocks stimulation due to metenkephalin.

minutes, the rate of sperm progression increased to 70% above that of the controls (Cariello and Nelson, 1984).

DISCUSSION

Responsiveness to the action of the pharmacological agents by sperm cells is quantitatively demonstrated in terms of changes in their rate (or pattern) of motile progression. We have here presented evidence that opioids and opiate antagonists elicit both time- and dose-dependent alterations in *Arbacia* sperm swimming rate. But it is quite evident that suspensions of living sperm cells of marine invertebrates, while sharing some physiological properties with the sperm of vertebrates yet exhibit characteristic behavioral responses that differ both qualitatively and quantitatively from other excitable tissues.

Intact sea urchin sperm cells respond to narcotics and antagonists. These experiments were undertaken following our preliminary observations that morphine had a pronounced effect in the sperm cells of the Mediterranean sea urchin, *Sphaerechinus granularis* (Cariello and Nelson, 1984). Quantitative determinations on the motility

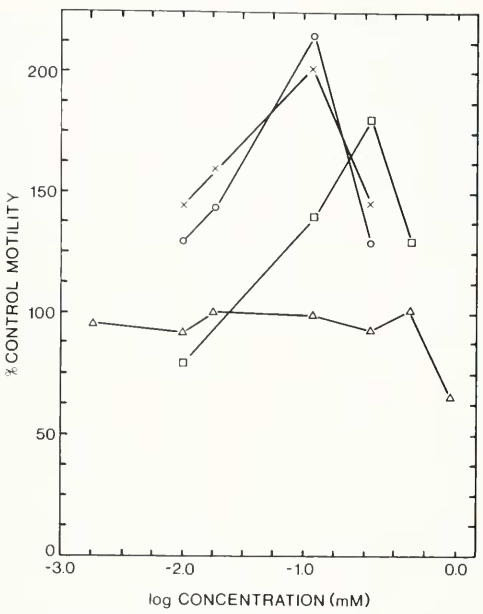

FIGURE 4. Plus-naloxone and *Arbacia* sperm. Abscissa: log conc. in m*M*. Ordinate: percent change in motility after 15-min incubation in ASW containing (+)-naloxone alone (open triangles); (−)-naloxone alone (open squares); metenkephalin alone (open circles); and metenkephalin and (+)-naloxone 0.005 m*M* (× - - - ×).

of sperm cells of *Arbacia punctulata* suspended in ASW showed that metenkephalin acetate caused a biphasic dose-dependent response (Fig. 1). Metenkephalin doubled the rate of progression measured after a 15-minute preincubation period, with lesser degrees of motility enhancement after shorter exposure. At concentrations greater than optimum, metenkephalin depressed motility more acutely with increasing exposure time. This aspect is brought into focus in the drug-interaction depicted in Figure 3 in which the subthreshold concentration of (−)-naloxone, having reversed metenkephalin's stimulatory effect at suboptimal amounts, becomes overridden allowing motility to decline as the metenkephalin was applied in supraoptimal doses. Under these conditions (−)-naloxone fulfills its putative role as a competitive opiate antagonist even though by itself it acts atypically in the sea urchin sperm system. Usually (−)-naloxone does not exhibit agonistic effects and so is considered to be a relatively pure antagonist which may be taken as an indicator for opioid receptor-mediated physiological processes and, biochemically, in ligand-displacement assays of naturally occurring morphine-like factors. While (−)-naloxone is almost devoid of agonistic effects in whole animal or organ-receptor assay, nevertheless it appears by virtue of the blocking of metenkephalin stimulation to be of the class of substances referred to either as partial agonists or agonist-antagonists in the context of the sea urchin sperm system.

The specificity of the sperm opioid receptor appears to be attested not only by (−)-naloxone's blocking of metenkephalin's agonist action, but further confirmed by the inability of (+)-naloxone to influence metenkephalin's stimulation as well as by the fact that (+)-naloxone itself is without discernible effect on *Arbacia* sperm motility at concentrations below 1 millimolar. According to Iijima *et al.* (1978), who synthesized

it from thebaine, the (+)-naloxone had no more than 1/1000 to 1/10,000 the pharmacological action of (−)-naloxone and can therefore "serve to test the stereospecificity of the biochemical and pharmacological actions of (−)-naloxone." These investigators tested the (+)-naloxone on three bioassay systems including the rat brain membrane receptor binding assay, guinea pig ileum, and the reversal of morphine inhibition of the adenylate cyclase activity of a neuroblastoma × glioma hybrid.

In the *Arbacia* sperm motility assay the threshold for metenkephalin stimulation fell in the micromolar range, that due to (−)-naloxone commenced at an order of magnitude higher, while above one millimolar, (+)-naloxone showed only a slightly depressant effect but did not reverse the metenkephalin effect at all. By these criteria, the *Arbacia* sperm may be considered to be endowed with opiate receptors that may bind endogenous opioids and also the opiate antagonist (−)-naloxone. Sastry and coworkers (1982) have extracted enkephalin-like and substance P-like compounds from human, rat, and bull spermatozoa, and from human seminal plasma and from rat male accessory glands which they detected by radioimmunoassay. The seminal plasma contained higher levels of met- and leu-enkephalins than did the sperm, while the distribution of substance P in these tissues was the reverse. These investigators point to the role of the opioid peptides in regulation of acetylcholine-induced Ca^{2+} fluxes which are essential for sperm motility and the acrosome reaction.

Immunoreactive β-endorphin-like material was detected in Leydig cells, epididymal epithelia, seminal vesicles, and vas deferens of rat, mouse, guinea pig, hamster, and rabbit (Shu-Dong *et al.,* 1982). Fravioli *et al.* (1984) investigated the possible role of β-endorphin, met-enkephalin, and calcitonin in human sperm motility regulation. In their study, they report that metenkephalin does not affect the motility at concentrations between nanomolar and millimolar, although β-endorphin starts to depress motility at about 0.5 micromolar. On the other hand, calcitonin, in seminal fluid at about three times its concentration in peripheral blood plasma, markedly depresses human sperm motility in the nanomolar range and completely inhibits movement at micromolar concentration. It is not clear at this time why (−)-naloxone behaves anomalously in stimulating *Arbacia* sperm, nor why metenkephalin's stimulatory effect on the sea urchin sperm should differ from its reported lack of effect on human sperm.

Similarly unanticipated results with opioids have been reported by Zagon and McLaughlin (1983, 1986). Heroin inhibition of the growth of transplanted neuroblastoma in mice was blocked by concomitant administration of naloxone. Paradoxically, when naloxone was tested alone at concentrations sufficient to interact with opiate receptors, this opiate antagonist was extremely effective in prevention or retardation of the tumor growth. According to these investigators, the antagonist naltrexone can promote tumorigenesis at a dose that prevents the analgesic action of morphine, but it exerts antineoplastic effects at concentrations that only temporarily block antinociception by morphine.

Conclusions

To meet criteria for pharmacological action, a given agent must elicit a characteristic response from sperm cells with appropriate dose- and time-dependent parameters. These may differ from responses observed in neurobiological systems: the dose required, the magnitude of altered action, the time course for the optimum expression should provide insights that could suggest the locus and number, as well as the accessibility, of specific receptors. Similar peculiarities have been documented in the behavior of sperm cells challenged by other well-defined agonists and antagonists.

The central action of narcotics and endogenous opioids has been attributed to

interference with neurotransmission since both morphine and β-endorphin inhibit the release of acetylcholine from brain synaptosomes by preventing Ca^{2+} uptake (Sanfacon *et al.,* 1977; Guerrero-Munoz *et al.,* 1979) and naloxone blocks these effects. This test system would serve as a relevant model for interprobation of sperm cell behavior assuming that the spermatozoa possessed opioid receptors with which appropriate ligands would interact to affect the calcium-dependent acetylcholine release mechanism. We have shown that: (1) the endogenous opioid metenkephalin in ASW alters the sperm cell swimming rate biphasically; (2) (−)-naloxone, the specific opiate antagonist, in a subthreshold dose reverses metenkephalin stimulation even though by itself, (−)-naloxone also exerts a dose-dependent biphasic response; and (3) (+)-naloxone is without effect in concentrations below 1 millimolar, both by itself and when combined with metenkephalin. Thus, it appears that *Arbacia* sperm cells may indeed be equipped with opioid receptors.

Alternatively, according to recently published reports by Haynes and Smith (1982) and Haynes *et al.* (1984), the opioid peptide β-endorphin and to a somewhat lesser extent, methionine enkephalin, inhibit motor endplate-specific acetylcholinesterase in skeletal muscle of embryonic and newborn rats.

In previous studies, it has been established that the sperm cells of *Arbacia punctulata* and other species possess a functional nicotonic cholinergic system which participates in regulation of motile performance. Calmodulin-antagonists, calcium-chelators, and calcium channel blockers also have been shown to alter the rate of motile progression (Cariello and Nelson, 1985). Moreover, eserine, nicotine, and decamethonium affect the uptake and intracellular distribution of calcium in sperm cells (Nelson *et al.,* 1982). In summary, the evidence is consistent with the presence in sperm cells of opioid receptors, although somewhat atypical, and also an interdependent acetylcholine-modulated calcium-uptake system. The nature and extent of interaction of these two systems in spermatozoa remain to be elucidated.

ACKNOWLEDGMENTS

This work has been partially supported by National Science Foundation Research Grants PCM8302582 and INT8300181 and by the Consiglio Nazionale di Ricerche. The authors wish to thank Dr. Giovanna Romano for her valuable assistance.

LITERATURE CITED

ATHERTON, R. W., E. W. RADANY, AND K. L. POLAKOSKI. 1978. Spectrophotometric quantitation of mammalian spermatozoon motility. 1. Human. *Biol. Reprod.* **18:** 624–628.

BAVISTER, B. D., A. F. CHEN, AND P. C. FU. 1979. Catecholamine requirement for hamster sperm motility *in vitro*. *J. Reprod. Fert.* **56:** 507–513.

BISHOP, M. R., B. V. R. SASTRY, D. E. SCHMIDT, AND R. D. HARBISON. 1976. Occurrence of choline acetyltransferase and acetylcholine and other quaternary ammonium compounds in mammalian spermatozoa. *Biochem. Pharmacol.* **25:** 1617–1622.

CARIELLO, L., AND L. NELSON. 1984. Enkephalin, opiate of the sperm cell. *Biol. Bull.* **167:** 540–541.

CARIELLO, L., AND L. NELSON. 1985. Transglutaminase inhibitors, calmodulin antagonists and calcium channel blockers: influence on *Arbacia* sperm motility. *Gamete Res.* **11:** 261–270.

CORNETT, L. E., AND S. MEIZEL. 1978. Stimulation of *in vitro* activation and the acrosome reaction of hamster sperm by catecholamines. *Proc. Natl. Acad. Sci. USA* **75:** 4954–4958.

EGBUNIKE, G. N. 1982. Effect of chloroquine on the motility and acetylcholinesterase activity of porcine spermatozoa during epididymal maturation. *Andrologia* **14**(6): 503–508.

FRAVIOLI, F., A. FABBRI, L. GNESSI, L. SILVESTRONI, C. MORRETTI, F. REDI, AND A. ISIDORI. 1984. Beta-endorphin, met-enkephalin and calcitonin in human semen: evidence for a possible role in human sperm motility. *Ann. N.Y. Acad. Sci.* **438:** 365–370.

GUERRERO-MUNOZ, F., M. DE L. GUERRERO, E. L. WAY, AND C. H. LI. 1979. Effect of β-endorphin on calcium uptake in the brain. *Science* **206:** 89–91.

HAYNES, L. W., AND M. E. SMITH. 1982. Selective inhibition of "motor-endplate specific" acetylcholinesterase by β-endorphin and related peptides. *Neuroscience* **7**: 1007–1013.

HAYNES, L. W., M. E. SMITH, AND D. G. SMYTH. 1954. Evidence for neurotrophic regulation of collagen-tailed acetylcholinesterase in immature skeletal muscle by β-endorphin. *J. Neurochem.* **42**: 1542–1551.

IIJIMA, I., J. MINAMIKAWA, A. E. JACOBSON, A. BROSSI, AND K. C. RICE. 1978. Studies in the (+)-morphinan series. 5. Synthesis and biological properties of (+)-naloxone. *J. Med. Chem.* **21**: 398–400.

NELSON, L. 1972. Neurochemical control of *Arbacia* sperm motility. *Exp. Cell Res.* **74**: 269–274.

NELSON, L. 1973. Preliminary evidence for cholinoreceptive sites in the excitability of spermatozoa. *Nature* **242**: 401–402.

NELSON, L. 1976. α-bungarotoxin binding by cell membranes. Blockage of sperm cell motility. *Exp. Cell Res.* **101**: 221–224.

NELSON, L. 1978. Chemistry and neurochemistry of sperm motility control. *Fed. Proc.* **37**: 2543–2547.

NELSON, L., M. E. GARDNER, AND M. J. YOUNG. 1982. Regulation of calcium distribution in bovine sperm cells: cytochemical evidence for motility control mechanisms. *Cell Motility* **2**: 225–242.

SANFACON, G., M. HOUDE-DEPUIS, R. VANIER, AND G. LABRECQUE. 1977. Calcium-induced modification of inhibition of acetylcholine release by morphine. *J. Neurochem.* **28**: 881–884.

SASTRY, B. V. RAMA, V. E. JANSON, AND A. K. CHATURVEDI. 1981. Inhibition of human sperm motility by inhibitors of choline acetyltransferase. *J. Pharm. Exp. Ther.* **216**: 378–384.

SASTRY, B. V. RAMA, V. E. JANSON, L. K. OWENS, AND O. S. TAYEB. 1982. Enkephalin-like and substance P-like immunoreactivities of mammalian sperm and accessory sex glands. *Biochem. Pharmacol.* **31**: 3519–3522.

SEKINE, T. 1951. Cholinesterase in pig spermatozoa. *J. Biochem.* **38**: 171–179.

SHU-DONG, T., D. M. PHILLIPS, N. HALMI, D. KRIEGER, AND C. W. BARDIN. 1982. β-endorphin is present in the male reproductive tract of five species. *Biol. Reprod.* **27**: 755–764.

STEWART, T. A., AND I. T. FORRESTER. 1978. Acetylcholinesterase and choline acetyltransferase in ram spermatozoa. *Biol. Reprod.* **19**: 271–279.

ZAGON, S., AND P. J. MCLAUGHLIN. 1983. Naltrexone modulates tumor response in mice with neuroblastoma. *Science* **221**: 671–673.

ZAGON, S., AND P. J. MCLAUGHLIN. 1986. Modulation of murine neuroblastoma in nude mice by opioid antagonists. In Press.

ZELLER, E. W., AND C. A. JOEL. 1941. Beitrage zur Fermentchemie des mannlichen Geschlectsapparates (2). *Helv. Chim. Acta* **24**: 968–976.